SIGNIFICANCE

Physical methods are poised to transform drug discovery and chemical biology by enabling true molecular design. While modeling work is already used extensively in drug discovery, its main role at present is to aid with idea generation or to filter large libraries of compounds for screening. Instead, we imagine using computational techniques extensively to guide the design process. Consider a medicinal chemist in the not-too-distant future who has just finished synthesizing several new derivatives of an existing inhibitor as potential drug leads targeting a particular biomolecule, and has obtained binding affinity or potency data against the desired biomolecular target. Before leaving work, he or she generates ideas for perhaps 100 new compounds which could be synthesized next, then sets a computer to work overnight prioritizing them. By morning, the compounds have all been prioritized based on reliable predictions of their affinity for the desired target, selectivity against alternative targets which should be avoided, solubility, and membrane permeability. The chemist then looks through the predicted properties for the top few compounds and selects the next ones for synthesis. If synthesizing and testing each compound takes several days, this workflow compresses roughly a year's work into a few days.

While this workflow is not yet a reality, huge strides have been made in this direction, with calculated binding affinity predictions now showing real promise [1–8], solubility predictions beginning to come online [9–11], and predicted drug resistance/selectivity also apparently tractable [12], with some headway apparent on membrane permeability [13, 14]. A considerable amount of science and engineering still remains to make this vision a reality, but, given recent progress, the question now seems more one of *when* rather than *whether*.

Recent progress in computational power, especially the widespread availability of graphics processing units (GPUs) and advances in automation [15] and sampling protocols, have helped simulation-based techniques reach the point where they now appear to have sufficient accuracy to be genuinely useful in guiding pharmaceutical drug discovery at least for a certain subset of problems [4–8, 16, 17]. Specifically, in some situations, free energy calculations appear to be capable of achieving RMS errors in the 1-2 kcal/mol range with current force fields, even in prospective applications. As a consequence, pharmaceutical companies are beginning to use these methods in discovery projects.

Unfortunately, these methods still have severe limitations, and a great deal of science and engineering is still needed before these techniques can achieve the major design impact we desire. For example, even "small" protein conformational conformational changes can yield errors up to 5 kcal/mol in calculated binding free energies [18], and force field limitations can pose major challenges as well [19]. Worse, the most important sources of error are not always clear.

Unfortunately, neither retrospective tests nor application of these techniques in drug discovery provides the necessary impetus and data to rapidly advance physical modeling techniques. While large-scale retrospective tests can be valuable for assessing how well we can currently do *retrospectively*, they are of limited value for paving the way forward or for assessing how well we will do for molecular design. Partly, retrospective tests allow for unintentional cheating, where a well-meaning researcher might apply a protocol they know will work in this particular situation where application in a design setting would lead to a different choice of protocol. For example, if the binding mode of a ligand is already known crystallographically, a researcher may use that binding mode in retrospective tests, whereas prospective or design work would require also selecting candidate binding modes, introducing substantial additional uncertainty [1, 20, 21]. This also means that in retrospective tests, researchers almost invariably try far fewer methods than in prospective tests, resulting in much less new insight. Prospective application, while important, also does not provide the necessary impetus, partly because often, the predicted compounds are in fact never tested [6] or the experimental data necessary to assess the quality of the predictions is absent – for example, because binding affinities are not measured or no crystallography is available.

To rapidly advance these methods, we need a series of community blind prediction challenges focused specifically on pushing the limits of predictive techniques, beginning from problems which are just barely tractable with today's methods and advancing to problems just past the frontier. These challenges should be designed to have precisely the necessary high quality experimental data, but also be prospective, predictive tests. While the Drug Design Data Resource (D3R [22], discussed further below) provides an existing community blind challenge on protein-ligand binding, it focuses on using *existing* pharmaceutical datasets for blind challenges, and not on introducing new data in a carefully controlled manner in order to maximize the learning value to the community [22]. In other words, D3R serves well to assess where we are now – but we need a carefully designed effort that will help the field achieve our goals.

One series of blind challenges, called "Statistical Assessment of Modeling of Proteins and Ligands" (SAMPL) provides a model we carry forward and extend here. SAMPL was begun by OpenEye software in 2007/2008 [23] at one of their CUP science events, and has run approximately every two years since then [24–31]; it transitioned to

being run by an outside group of academics beginning partially with SAMPL3 in 2012, then completely for SAMPL4 (2014) and SAMPL5 (2016), with the PI of this proposal playing a key organizational role in SAMPL3-SAMPL5. SAMPL is modeled as a *challenge* rather than a *competition*, with a key goal being to maximize what is learned rather than to declare winners and losers, with the idea that learning will provide the greatest long-term rewards in terms of progress in the field. SAMPL has always involved a component focused on calculation of relatively straightforward physical properties such as hydration free energies, but also introduced host-guest systems as model binding systems for SAMPL3-5, supplementing protein-ligand challenges (on trypsin and HIV integrase) which appeared in SAMPL3-4. SAMPL has already been a tremendous resource for the community, resulting in roughly 100 publications (some are coming out as of this writing) which are typically cited 5-50 times or more each [refs].

Here, we continue the legacy of SAMPL, but design a new series of SAMPL challenges specifically to maximize learning value to the community. Until now, this has been impossible, because SAMPL has been entirely unfunded, so its existence has required "donation" of data from various sources rather than data collected specifically to drive improvements to modeling. Funding of SAMPL will also allow SAMPL to deliberately bridge the gap between calculations of simple physical properties like hydration – which can be calculated fairly accurately with today's methods [28] – and the D3R Grand Challenges on protein-ligand binding, which are a major source of consternation for the community so far [22, 32–34]. Unless this gap is bridged, there is the very real possibility that modeling may simply continue to fall far short of expectations in pharmaceutical challenges like D3R for reasons which are unclear. The extension of SAMPL proposed here will allow us to form blind challenges designed to highlight major reasons for failure and drive progress towards resolving them.

Our major goal is to rapidly advance predictive modeling to where it can guide experimental work doing biomolecular design, and extension of the SAMPL challenges will do exactly that. The Computer Aided Structure Prediction (CASP) series of competitions provide an example of how a focused effort can realistically have a dramatic impact on predictive modeling in a relatively short space of time. **[JDC to write text here]** While our model for the SAMPL challenges is slightly different than that for CASP (for example, we have less emphasis on competition) we see SAMPL playing the same key role in driving predictive modeling, but in our field of protein-ligand interactions. In our view it will play a vital role in enhancing the work being done on *existing* data by D3R.

INNOVATION

Blind predictive challenges – and SAMPL in particular – already play a key role in fostering innovation in the field, especially in the form of method development, testing, and hardening, as well as force field development. Thus, this work, by continuing and extending SAMPL, will advance innovative new science. It is worth briefly highlighting several historical examples, though far more are available in the SAMPL literature. The first several SAMPL challenges on hydration free energies had rather hit-and-miss performance, highlighting pitfalls of existing methods and force fields which led to marked improvements in PB models [refs], recognition of some limitations of fixed-charge force fields [refs], repair of some of these force field deficiencies via additional polarization or introduction of off-site charges [refs], and helped motivate alternate implicit or hybrid solvent models [ref]. Together these advances led to a marked improvement in accuracy for calculations of hydration free energies between SAMPLX and SAMPLY (Figure []). Shifts in protonation state and tautomer proved particularly important in the recent SAMPL5 $\log D$ challenge[refs], as they presumably will be for protein-ligand binding as well. Host-guest binding studies have also been particularly important[ref benchmark sets], highlighting the importance of salt effects [refs] and in some cases revealing more severe force field limitations than observed in hydration and distribution challenges [ref Gilson group stuff], pointing the way forward for improving predictive models of molecular interactions [ref].

This work is also innovative because of the uniqueness of SAMPL. While there are other predictive challenges in the area of biomolecular modeling, such as D3R [ref], the pKa cooperative [ref], and CASP [ref], none are specifically focused on driving quantitatively predictive protein-ligand modeling. SAMPL is unique because – at least with the deliberate design plan we propose here – it will be specifically tailored in order to drive improvements in modeling for biomolecular design. It also plays an enabling role for a wide variety of other science, rather than functioning as a stand-alone entity. SAMPL benefits the whole modeling community – for example, even docking software has improved from SAMPL hydration challenges [ref Coleman], and even commercial software has introduced new features and made improvements based on SAMPL challenges [cite Klamt stuff]. In effect, SAMPL serves as an innovation engine, and this work will ensure that SAMPL not only continues but becomes even more valuable to the community.

This work also focuses on innovative experimental methods. Specifically, in Aim 3, we are developing new, high-throughput experiments for studying and measuring protein ligand binding, with careful automated error analysis,

and a heavy emphasis on robotics and automation. The Chodera lab – responsible for Aim 3 – already has substantial expertise in the area of automation of experiments and better uncertainty analysis of those experiments. Additionally, they design their own 3D printed parts to facilitate these experiments, and make plans for these available to others. Thus this work is at the forefront of innovation in high-throughput, automated experiments to produce high quality data with well characterized uncertainties. Not only will the data itself be of prime importance to the community, but the techniques themselves will help future experimental work.

In Aim 4, we will not only run SAMPL community challenges, but also perform our own reference calculations with the latest techniques, testing their accuracy and using these to assess the current state-of-the-art. Both the Mobley and Chodera labs are experts in development of free energy methods for application to physical properties (e.g.. [a couple refs]) and binding (e.g. [a couple refs]), and the reference calculations we perform in Aim 4 are particularly important for innovation as well, serving several key roles: (1) Benchmarking our latest method developments against current "best practices" methods (by doing calculations via both approaches); (2) Facilitating learning, allowing others to experiment with how a change in method or force field impacts results; (3) Focusing the field on key issues by doing sensitivity analysis to whether conditions such as ionic strength, protonation state, tautomer choice, etc. impact computed values.

Innovation in Aim 4 extends beyond reference calculations to analysis of the challenge itself. When methods differ in performance, it is critical to understand whether the differences are statistically significant and important, and to provide an accurate accounting of the uncertainty in performance measures. Thus, careful and innovative analysis of challenge outcomes is particularly important in SAMPL [refs], in some cases driving experimentation with new performance metrics [refs]. Additionally, we try to draw attention to and promote analysis of model uncertainty (as distinct from statistical uncertainty) in calculated values [refs], as understanding the confidence levels of predictions is particularly important for guiding molecular design.

APPROACH

Our approach to systematically advancing modeling for biomolecular design involves collecting carefully tailored experimental data for challenges focusing on physical property prediction, host-guest binding, and biomolecular binding. Thus, we have four main aims, three of which focus on tailoring and generating this experimental data, and a fourth which focuses on running SAMPL challenges to advance modeling and maximize the learning value to the community. These SAMPL challenges will run yearly (though not necessarily every aspect of the proposal will feature in every SAMPL challenge), so each of the aims focused on generating experimental data has a series of stages which correspond to individual future SAMPL challenges.

Aim 1: Generate new data for "simple" SAMPL blind challenges on physical property prediction. Our first aim focuses on generating solution-phase physical property data for small, drug-like molecules – essentially continuing the tradition begun with hydration free energies in SAMPL0-4 and continued with water-cyclohexane distribution coefficients in SAMPL5. Distribution coefficients proved tremendously valuable to the community in SAMPL5 for a number of reasons, highlighting a number of key issues where modeling needs to improve, so they will form the basis for the physical property component in SAMPL6 and return in several subsequent SAMPL challenges.

Distribution coefficients proved to be precisely the right level of difficulty for a SAMPL challenge focused on maximizing learning. Specifically, they were challenging enough that many methods performed poorly, with even the best methods having accuracies less than would have been expected based on their ability to calculate hydration free energies in water [35]. At the same time, methods typically did well enough that it was possible to learn a great deal from examining failure, and the major sources of error were issues which will also plague prediction of ligand-receptor interactions. These included neglect of changes in protonation state on transfer between environments, uncertainty as to the relevant protonation state and/or tautomer in one or both environments [35], problems with sampling the conformation of some of the larger ligands [35, 36], and force field limitations [37]. Our partnership with Genentech for these measurements also meant that the compounds were from Genentech's library and thus very drug-like, unlike typical compounds seen in hydration free energy challenges of the past. In some respects, distribution coefficients posed the ideal SAMPL challenge, hitting the sweet spot in terms of difficulty – difficult enough that clear failures were frequent and there is much room for improvement, but not so difficult that the reasons for failure were unclear in general. Still, the challenge could have been improved by follow-up experiments to re-check some of the experimental results [30, 37–39] – but without funding for someone to continue working in this space, this has so far been impossible.

Because distribution coefficients were so valuable in SAMPL5 and are driving so much needed method development [refs], we will measure new cyclohexane-water distribution coefficients for our first data set here and this will form the basis for the physical property component of SAMPL6. Because octanol-water distribution coefficients are also

potentially tractable [35], and measured much more frequently experimentally, we plan to generate measurements for the same compound distributing between both cyclohexane-water and octanol-water for this challenge.

However, distribution coefficients conflate several issues which are still complex, namely protonation state and tautomer prediction, as well as transfer into different environments. Thus, we will likely need to turn to separating these issues to improve our handling of them one at a time. For SAMPL7, then, our tentative plan is to measure pKa values for an extensive set of drug-like molecules in water and provide data specifically on pKa values, thereby separating the issues of predicting protonation state from those of transfer.

In the next challenge, SAMPL8, we would re-combine the pKa and transfer issues in a way to maximize learning – specifically, we will not only measure distribution coefficients but also measure pKa values for the same set of compounds, allowing participants to (a) predict distribution; (b) predict pKa; and (c) predict partitioning.

Several other avenues are of interest for future datasets as well, especially for SAMPL9 and SAMPL10. New computational techniques are targeting membrane permeability [13, 14], and this is experimentally accessible (see support letters from Pfizer and Merck), leading to potential interest in new datasets and challenges focused there. Alternatively, partition/distribution into other environments aside from cyclohexane or octanol may provide significant value, especially given dielectric constant issues posed by current force fields [ref]. Solubility measurements may also be suitable for a late-stage SAMPL challenge such as SAMPL10, since solubility predictions are now beginning to become tractable [9–11] (with Schrödinger also working on amorphous solubility prediction).

Here, our experimental work will be performed with partners in the pharmaceutical industry, following roughly the model used for SAMPL5, where we partnered with Genentech, sending Bas Rustenburg, a graduate student in the Chodera lab, to conduct measurements of $\log D$ using existing equipment. We find that pharma often has substantial access to equipment – such as the expensive Sirius T3 for measuring partition, distribution, and pKa – which is very hard to find in academia, and it may not always see heavy use. Thus, sending a person into pharma to generate data can be an extremely fruitful line of inquiry, as the relevant equipment and expertise is already present but manpower is lacking. Here, we are partnering with Genentech, Pfizer, and Merck (see support letters), who have agreed to give us access to the equipment and expertise we need for the necessary measurements. This work will provide funding for us to send a joint experiment and modeling student from the Mobley group to conduct the relevant measurements with our pharma partners. As noted, we already know that this model will work given our experience in SAMPL5 [39], and our pharma partners see the value of this data and the SAMPL challenge to the modeling community.

This new experimental data is critical to make SAMPL what it ought to be. While the community has already benefitted tremendously from SAMPL, as indicated by the tremendous number of publications, the citation count, and lessons learned, the benefits are not what they could have been if the effort had funding. For example, we planed to ensure that the measured $\log D$ values in SAMPL5 spanned the full dynamic range allowed by the experiment with roughly equal populations at all values of $\log D$, but because our experimental work ended when the relevant Genentech internship did, this was not accomplished [30, 39]. With funding, we will be able to continue experiments work until the necessary data is collected rather than terminating it at a specific time point dictated by industry funding. This snafu had downstream consequences in that many participants observed that calculated values spanned a larger dynamic range than the experimental values, but it is unclear if this is an artifact of the data set measured (which has a relatively limited dynamic range), or because of issues with the calculations or with the experiments themselves [30, 37–39]. With more dynamic range available in the experimental data, and the ability to do follow-up experiments (as we will have here) this would have been resolved, providing further impetus to improve models.

Thus, in Aim 1, we will generate a range of new, high quality physical property data on distribution, partition, pKa prediction, and membrane permeability to facilitate community learning via a series of new SAMPL challenges which are the focus of Aim 4 below. This will extend the prior success of SAMPL challenges on hydration and distribution between water and cyclohexane, focusing the community on problems that are of tremendous relevance to accurately predicting biomolecular interactions but without the additional complexity introduced by simulations of proteins or nucleic acids. This data thus paves the way to applications in more complex systems such as those addressed in Aims 2 and 3.

Aim 2: Measure binding of novel host-guest complexes for introductory ligand binding challenges.

Physical property challenges focus on the behavior of small molecules and how this is modulated by environment, introducing a number of issues relevant to drug discovery – but binding in host-guest systems introduces a wider variety of challenges relevant to biomolecular interactions, but without the full array of challenges seen in protein-ligand interactions, as reviewed recently [**?**]. Thus, we believe that new data and SAMPL challenges on

host-guest binding are a critical step towards accurately modeling biomolecular interactions. Already, over the past several SAMPL challenges, host-guest systems have provided key tests for modeling of binding interactions, driving a great deal of learning about challenges such as how co-solvents and protonation modulate binding and the limitations or pitfalls of different methods for calculating binding free energies [**?**, 29, 31, 40]. Host-guest binding proves remarkably difficult to model accurately [41], probably in part due to force field limitations – itself an important insight and one which is leading to new developments [42]. Here, we design a series of SAMPL challenges focused on two general classes of host-guest systems – cucubiturils and derivatives, and deep-cavity cavitands – both of which build in studies from prior SAMPLs.

Cucubituril-based receptors as model binding systems

Cucubituril derivatives for host-guest binding. Building on previous success with cucubit[n]uril experiments for SAMPL challenges [43, 44, 62], we will conduct a series of new experiments on these receptors for three new SAMPL challenges, with experimental work conducted by co-investigator Isaacs, an expert on these systems. Cucurbit[n]uril receptors are particularly well suited for the SAMPL challenges because they exhibit: 1) high binding constants toward suitable guests in water (routinely μM to nM; occasionally pM to fM) [45–51], 2) high selectivities between structurally related guests which translate into large $\Delta\Delta G$ values [52], 3) low molecular weights (1000-2000 amu) which allow high levels of theory to be used, and 4) limited conformational degrees of freedom. Thus, for SAMPL, we will resynthesize a series of CB[n]-type receptors of increasing complexity, measure Ka values and determine host-guest stoichiometry and geometry toward biologically relevant guests in order to stringently test methods for predicting binding. Figure 1 shows the chemical structures of three hosts – Me4CB[8] [53], glycoluril hexamer [54], and acyclic CB[n]-type receptors [55–60] which span the range from preorganized macrocyclic host to uncharged acyclic but preorganized host to highly charged acyclic host.

drug	features
memantine	adamantane; 1:1
saxagliptin	adamantane; 1:1
premarin	steroid
pancuronium	steroid
varenicline	1:1 vs 1:2
valsartan	pKa 4.37
omeprazole	pKa 4.77
ranolazine	pKa 7.17; epitopes
pradaxa	pKa 3.87; epitopes
nilotinib	epitopes; pKa 6.3
sensipar	epitopes; folding
vyvance	diamine; epitopes; folding
minocycline	tetracyclin; amino aniline

Table 1: Selected drugs as guests

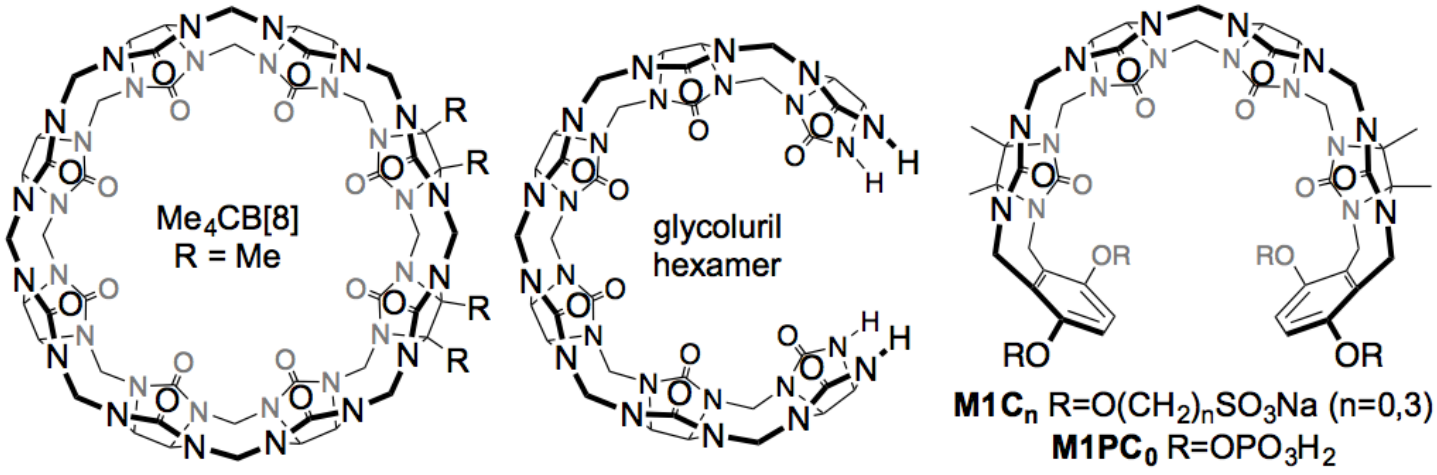

Figure 1: **Structures of Me$_4$CB[8], glycouril hexamer, and acyclic CB[n]-type receptors.**

SAMPL6, 8 and 10 cucubituril challenges. *For SAMPL6*, we will measure K_a and ΔH values, stoichiometry, and geometry for the interaction of Me4CB[8] (a soluble CB[8] derivative) with 15 guests (selected top drugs, Table CB1) by either direct or competition isothermal titration calorimetry (ITC), UV/Vis or fluorescence indicator displacement assay, or NMR competition experiments, as previously [45, 46, 61, 62]. Our selection of Me$_4$CB[8] binding to top drugs allows us to modulate the computational complexity by: 1) changing host flexibility (e.g. Me$_4$CB[8] can exhibit ellipsoidal deformation) [53], 2) allowing the possibility of binary or ternary (e.g. 1:1 and/or 1:2 host:guest) complexes [63–65], 3) using drugs with several potential binding epitopes to induce sampling issues. Host:guest stoichiometry and geometry (e.g. which binding epitope is complexed) will be addressed by

ITC "n" values, Job plots monitored by UV/Vis or NMR [66], and by 1H NMR complexation induced changes in chemical shifts [67]. All three sets of studies will be conducted in phosphate buffered saline (pH 7.4 with physiological salt) which introduces its own complexities due to salt competition for binding [**?**, 68]. For *SAMPL8*, we will focus on binding of the same 15 drugs (Table 1), but to glycoluril hexamer. This is an ideal next step as this host exhibits increased conformational dynamics, and influences the number and energy of solvating (and unusually coordinated) water molecules implicated in the high binding constants for CB[n]-guest complexes [51, 69]. The selected drugs include several with pKa values in the 3.8 to 7.4 range; given that CB[n]-type receptors (like biomolecular receptors) can induce pKa shifts in their guests of up to 4 pKa units [70–72], this will test how well models can predict these effects. Additionally, it will couple nicely with the focus on pKa values in Aim 1. *SAMPL10* will shift to acyclic CB[n]-type receptors (e.g. M1C_3, M1C_0, and M1PC_0 that contain anionic solubilizing groups attached via different linker lengths. As in SAMPL2, these acyclic CB[n]-type receptors introduce conformational complexity, and water interactions play a key role. Moreover, the presence of 4 anionic groups in close proximity to the cavity are expected to have a significant influence on the balance between ion-dipole interactions and the solvation of the free host.

Gibb deep cavity cavitands for host-guest studies

History of octa-acid SAMPL challenges. During SAMPL4 [73] and SAMPL5 [74] we focused on two hosts: the octa-acid 1 (R = H) and another octa-acid derivative with four methyl groups positioned at the portal of the binding pocket (1, R = Me). These studies used Isothermal Titration Calorimetry (ITC) to measure the thermodynamics of (1) host 1 (R = H) complexing a range of carboxylate guests, and (2) the binding of carboxylate and trimethylammonium guests to both hosts (1, H = H and Me). In both cases ^{1}H-NMR titration was also used in a confirmatory role for ITC-derived free energies of binding. SAMPL5 emphasized how differences in the shape of the hydrophobic pocket of the host can have a profound affect on affinity for some guests.

Novel deep cavity hosts probe the effects of binding site charge constellations. For future SAMPL challenges, we will expand on the range of hosts by including 2 and 3 in our ITC studies. Like cavitand 1, host 2 is an octa-acid derivative. However, the four benzoate groups are relocated from the extreme exterior in the case of 1, to the rim of the binding pocket in 2. We surmise that this will have a direct effect on the binding of charged guests, but more subtly, an indirect effect on guest complexation via changes to the solvation of the empty host. Octa-trimethylammonuim cavitand ("positand" 3) has the same overall architecture as host 1, but inverts the charges on the water solubilizing exterior coat. While it is not yet clear if this switch in groups relatively remote from the pocket will directly affect guest complexation, results from related systems suggest it can (unpublished).

Guests for the five proposed ITC studies will be obtained from commercial sources, focusing on molecules that probe the limitations of current force-fields as well as new data as it is gathered.

SAMPL6-10 deep cavity cavitand challenges. The host-guest challenge for SAMPL6 will focus on how well the effect of host carboxylate substituent location can be predicted, and will involve hosts 1 and 2 with a set of five, previously uninvestigated guests. SAMPL7 will provide a second iteration of this experiment to test algorithmic improvements in predictive modeling following SAMPL6 by comparing hosts 1 and 3 with a different set of guests. We anticipate that because of the relative remoteness of the charged groups in these two hosts, the effects of switching charges will be subtler than the differences between 1 and 2. SAMPL8 will consider the effect of common biologically-relevant counterions/salts salts on guest binding, comparing the effects of NaCl and NaI on the complexation of five guests to 1. We have previously shown that iodide has a weak affinity for the binding pocket of 1, whilst sodium ions have an affinity for the outer carboxylates [75], requiring modeling to capture the differential affinities of these ions in addition to guest affinities to successfully model the observed affinities. SAMPL9 will follow up on this by examining the effects of these same two salts on the complexation of five guests to 3, again giving the modeling community time to incorporate algorithmic improvements following SAMPL8. While we have not yet quantified salt affinities to host 3, we expect the iodide to have affinity for both the pocket and the positively charged solubilizing groups. For SAMPL10 we will consider the effects of co-solvents on the binding of five guests to 1 and 2 to probe the effect of co-solvent competition for the binding site, as well as effects cosolvents may have in weakening the hydrophobic effect.

Aim 3. Generate biologically relevant advanced model systems for protein-ligand binding challenges. We will identify suitable biological protein-ligand model systems (difficult but tractable in order to push the limits of physical techniques) then measure binding and develop these for blind challenges. This will include binding studies on human serum albumin and bromodomains or aspartyl proteases; initial binding data will be expanded by the selection of additional ligands or the creation of mutations in the protein that modulate binding.

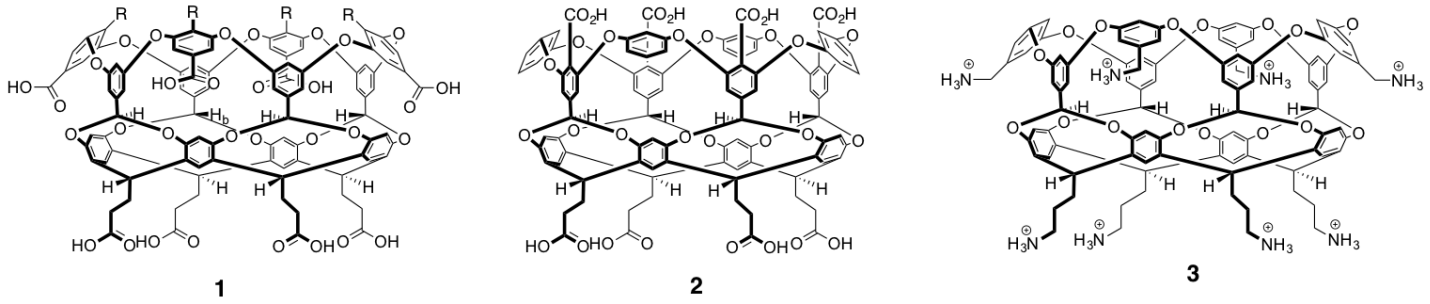

Figure 2: **Gibb deep cavity cavitands for SAMPL6-10.**

Aim 4. Coordinate, run, and analyze blind challenges to advance modeling of binding. The data collected in Aims 1-3 will drive annual SAMPL blind challenges, allowing the field to test the latest methods and force fields to assess progress, compare them against one another head-to-head, and perform sensitivity analysis to learn how much different factors (protonation state, tautomer selection, solvent model, force field, sampling method, etc.) affect predictive power. Results will then feed back into improved treatment of these factors for subsequent challenges, driving regular cycles of application, learning, and advancement.

TIMELINE

COLLABORATION MANAGEMENT PLAN

OUTLOOK

Bibliography and References Cited

[1] Mobley, D. L. and Klimovich, P. V.: Perspective: Alchemical free energy calculations for drug discovery. J. Chem. Phys. 137(23): 230901, January 2012.

[2] Christ, C. D. and Fox, T.: Accuracy Assessment and Automation of Free Energy Calculations for Drug Design. J. Chem. Inf. Model. 54(1): 108–120, January 2014.

[3] Deng, N., Forli, S., He, P., Perryman, A., Wickstrom, L., Vijayan, R. S. K., Tiefenbrunn, T., Stout, D., Gallicchio, E., Olson, A. J., and Levy, R. M.: Distinguishing Binders from False Positives by Free Energy Calculations: Fragment Screening Against the Flap Site of HIV Protease. J. Phys. Chem. B. 119(3): 976–988, January 2015.

[4] Sherborne, Bradley, : Opening the lid on FEP. J Comput Aided Mol Des. 2016.

[5] Wang, L., Wu, Y., Deng, Y., Kim, B., Pierce, L., Krilov, G., Lupyan, D., Robinson, S., Dahlgren, M. K., Greenwood, J., Romero, D. L., Masse, C., Knight, J. L., Steinbrecher, T., Beuming, T., Damm, W., Harder, E., Sherman, W., Brewer, M., Wester, R., Murcko, M., Frye, L., Farid, R., Lin, T., Mobley, D. L., Jorgensen, W. L., Berne, B. J., Friesner, R. A., and Abel, R.: Accurate and Reliable Prediction of Relative Ligand Binding Potency in Prospective Drug Discovery by Way of a Modern Free-Energy Calculation Protocol and Force Field. J Am Chem Soc. 137(7): 2695–2703, February 2015.

[6] Christ, C. D. Binding affinity prediction from molecular simulations: A new standard method in structure-based drug design?, May 2016.

[7] Cui, G. Affinity Predictions with FEP+: A Different Perspective on Performance and Utility, May 2016.

[8] Verras, A. Free Energy Perturbation at Merck: Benchmarking against Faster Methods, May 2016.

[9] Schnieders, M. J., Baltrusaitis, J., Shi, Y., Chattree, G., Zheng, L., Yang, W., and Ren, P.: The Structure, Thermodynamics, and Solubility of Organic Crystals from Simulation with a Polarizable Force Field. J. Chem. Theory Comput. 8(5): 1721–1736, May 2012.

[10] Park, J., Nessler, I., McClain, B., Macikenas, D., Baltrusaitis, J., and Schnieders, M. J.: Absolute Organic Crystal Thermodynamics: Growth of the Asymmetric Unit into a Crystal via Alchemy. J. Chem. Theory Comput. 10(7): 2781–2791, July 2014.

[11] Liu, S., Cao, S., Hoang, K., Young, K. L., Paluch, A. S., and Mobley, D. L.: Using MD Simulations To Calculate How Solvents Modulate Solubility. Journal of Chemical Theory and Computation. 12(4): 1930–1941, February 2016.

[12] Leonis, G., Steinbrecher, T., and Papadopoulos, M. G.: A Contribution to the Drug Resistance Mechanism of Darunavir, Amprenavir, Indinavir, and Saquinavir Complexes with HIV-1 Protease Due to Flap Mutation I50V: A Systematic MM–PBSA and Thermodynamic Integration Study. J. Chem. Inf. Model. 53(8): 2141–2153, August 2013.

[13] Lee, C. T., Comer, J., Herndon, C., Leung, N., Pavlova, A., Swift, R. V., Tung, C., Rowley, C. N., Amaro, R. E., Chipot, C., Wang, Y., and Gumbart, J. C.: Simulation-Based Approaches for Determining Membrane Permeability of Small Compounds. J. Chem. Inf. Model. 56(4): 721–733, April 2016.

[14] Comer, J., Schulten, K., and Chipot, C.: Calculation of Lipid-Bilayer Permeabilities Using an Average Force. J Chem. Theory Comput. 10(2): 554–564, February 2014.

[15] Liu, S., Wu, Y., Lin, T., Abel, R., Redmann, J. P., Summa, C. M., Jaber, V. R., Lim, N. M., and Mobley, D. L.: Lead optimization mapper: Automating free energy calculations for lead optimization. J Comput Aided Mol Des. 27(9): 755–770, September 2013.

[16] Mikulskis, P., Genheden, S., and Ryde, U.: A Large-Scale Test of Free-Energy Simulation Estimates of Protein–Ligand Binding Affinities. J. Chem. Inf. Model. 54(10): 2794–2806, October 2014.

[17] Homeyer, N., Stoll, F., Hillisch, A., and Gohlke, H.: Binding Free Energy Calculations for Lead Optimization: Assessment of Their Accuracy in an Industrial Drug Design Context. J. Chem. Theory Comput. 10(8): 3331–3344, August 2014.

[18] Lim, N. M., Wang, L., Abel, R., and Mobley, D. L.: Sensitivity in binding free energies due to protein reorganization. Journal of Chemical Theory and Computation. July 2016.

[19] Rocklin, G. J., Boyce, S. E., Fischer, M., Fish, I., Mobley, D. L., Shoichet, B. K., and Dill, K. A.: Blind Prediction of Charged Ligand Binding Affinities in a Model Binding Site. J. Mol. Biol. 425(22): 4569–4583, November 2013.

[20] Mobley, D. L., Graves, A. P., Chodera, J. D., McReynolds, A. C., Shoichet, B. K., and Dill, K. A.: Predicting absolute ligand binding free energies to a simple model site. J. Mol. Biol. 371(4): 1118–1134, August 2007.

[21] Boyce, S. E., Mobley, D. L., Rocklin, G. J., Graves, A. P., Dill, K. A., and Shoichet, B. K.: Predicting ligand binding affinity with alchemical free energy methods in a polar model binding site. J. Mol. Biol. 394(4): 747–763, December 2009.

[22] Gathiaka, S., Liu, S., Chiu, M., Yang, H., Stuckey, J. A., Kang, Y. N., Delproposto, J., Dunbar, J. B., Carlson, H. A., Burley, S., Walters, W., Amaro, R. E., Feher, V., and Gilson, M. K.: D3R Grand Challenge 2015: Evaluation of Protein-Ligand Pose and Affinity Prediction. J Comput Aided Mol Des. 2016.
[23] Nicholls, A., Mobley, D. L., Guthrie, J. P., Chodera, J. D., Bayly, C. I., Cooper, M. D., and Pande, V. S.: Predicting Small-Molecule Solvation Free Energies: An Informal Blind Test for Computational Chemistry. J. Med. Chem. 51(4): 769–779, February 2008.
[24] Nicholls, A., Wlodek, S., and Grant, J. A.: The SAMP1 Solvation Challenge: Further Lessons Regarding the Pitfalls of Parametrization. J. Phys. Chem. B. 113(14): 4521–4532, April 2009.
[25] Mobley, D. L., Bayly, C. I., Cooper, M. D., and Dill, K. A.: Predictions of Hydration Free Energies from All-Atom Molecular Dynamics Simulations. J Phys Chem B. 113: 4533–4537, January 2009.
[26] Geballe, M. T., Skillman, A. G., Nicholls, A., Guthrie, J. P., and Taylor, P. J.: The SAMPL2 blind prediction challenge: Introduction and overview. J Comput Aided Mol Des. 24(4): 259–279, May 2010.
[27] Geballe, M. T. and Guthrie, J. P.: The SAMPL3 blind prediction challenge: Transfer energy overview. J Comput Aided Mol Des. 26(5): 489–496, April 2012.
[28] Mobley, D. L., Wymer, K. L., Lim, N. M., and Guthrie, J. P.: Blind prediction of solvation free energies from the SAMPL4 challenge. J Comput Aided Mol Des. 28(3): 135–150, March 2014.
[29] Muddana, H. S., Fenley, A. T., Mobley, D. L., and Gilson, M. K.: The SAMPL4 host–guest blind prediction challenge: An overview. J Comput Aided Mol Des. 28(4): 305–317, March 2014.
[30] Bannan, C. C., Burley, K. H., Chiu, M., Shirts, M. R., Gilson, M. K., and Mobley, D. L.: Blind prediction of cyclohexane-water distribution coefficients from the SAMPL5 challenge. 2016.
[31] Yin, J., Henriksen, N. M., Slochower, D. R., Chiu, M. W., Mobley, D. L., and Gilson, M. K.: Overview of the SAMPL5 Host-Guest Challenge: Are We Doing Better? J Comput Aided Mol Des. 2016.
[32] Ignjatović, M. M., Caldararu, O., Dong, G., Muñoz-Gutierrez, C., Adasme-Carreño, F., and Ryde, U.: Binding-affinity predictions of HSP90 in the D3R Grand Challenge 2015 with docking, MM/GBSA, QM/MM, and free-energy simulations. J Comput Aided Mol Des. pp 1–24, August 2016.
[33] Deng, N., Flynn, W. F., Xia, J., Vijayan, R. S. K., Zhang, B., He, P., Mentes, A., Gallicchio, E., and Levy, R. M.: Large scale free energy calculations for blind predictions of protein–ligand binding: The D3R Grand Challenge 2015. J Comput Aided Mol Des. pp 1–9, August 2016.
[34] Sunseri, J., Ragoza, M., Collins, J., and Koes, D. R.: A D3R prospective evaluation of machine learning for protein-ligand scoring. J Comput Aided Mol Des. pp 1–11, September 2016.
[35] Bannan, C. C., Calabró, G., Kyu, D. Y., and Mobley, D. L.: Calculating Partition Coefficients of Small Molecules in Octanol/Water and Cyclohexane/Water. Journal of Chemical Theory and Computation. 12(8): 4015–4024, August 2016.
[36] Luchko, T., Blinov, N., Limon, G. C., Joyce, K. P., and Kovalenko, A.: SAMPL5: 3D-RISM partition coefficient calculations with partial molar volume corrections and solute conformational sampling. J Comput Aided Mol Des. pp 1–13, September 2016.
[37] Paranahewage, S. S., Gierhart, C. S., and Fennell, C. J.: Predicting water-to-cyclohexane partitioning of the SAMPL5 molecules using dielectric balancing of force fields. J Comput Aided Mol Des. pp 1–7, August 2016.
[38] Klamt, A., Eckert, F., Reinisch, J., and Wichmann, K.: Prediction of cyclohexane-water distribution coefficients with COSMO-RS on the SAMPL5 data set. J Comput Aided Mol Des. pp 1–9, July 2016.
[39] Rustenburg, A. S., Dancer, J., Lin, B., Feng, J. A., Ortwine, D. F., Mobley, D. L., and Chodera, J. D.: Measuring experimental cyclohexane-water distribution coefficients for the SAMPL5 challenge. bioRxiv. 063081 pp, July 2016.
[40] Bhakat, S. and Söderhjelm, P.: Resolving the problem of trapped water in binding cavities: Prediction of host-guest binding free energies in the SAMPL5 challenge by funnel metadynamics. J Comput Aided Mol Des. 2016.
[41] Henriksen, N. M., Fenley, A. T., and Gilson, M. K.: Computational Calorimetry: High-Precision Calculation of Host–Guest Binding Thermodynamics. Journal of Chemical Theory and Computation. 11(9): 4377–4394, September 2015.
[42] Yin, J., Fenley, A. T., Henriksen, N. M., and Gilson, M. K.: Toward Improved Force-Field Accuracy through Sensitivity Analysis of Host-Guest Binding Thermodynamics. The Journal of Physical Chemistry B. 119(32): 10145–10155, August 2015.
[43] Ma, D., Glassenberg, R., Ghosh, S., Zavalij, P. Y., and Isaacs, L.: Acyclic cucurbituril congener binds to local anaesthetics. Supramolecular Chemistry. 24(5): 325–332, May 2012.
[44] Cao, L. and Isaacs, L.: Absolute and relative binding affinity of cucurbit[7]uril towards a series of cationic guests. Supramolecular Chemistry. 26(3-4): 251–258, March 2014.
[45] Cao, L., Šekutor, M., Zavalij, P. Y., Mlinarić-Majerski, K., Glaser, R., and Isaacs, L.: Cucurbit[7]uril·Guest Pair

with an Attomolar Dissociation Constant. Angew. Chem. Int. Ed. 53(4): 988–993, January 2014.
[46] Liu, S., Ruspic, C., Mukhopadhyay, P., Chakrabarti, S., Zavalij, P. Y., and Isaacs, L.: The Cucurbit[n]uril Family: Prime Components for Self-Sorting Systems. Journal of the American Chemical Society. 127(45): 15959–15967, November 2005.
[47] Mock, W. L. and Shih, N. Y.: Structure and selectivity in host-guest complexes of cucurbituril. The Journal of Organic Chemistry. 51(23): 4440–4446, November 1986.
[48] Assaf, K. I. and Nau, W. M.: Cucurbiturils: From synthesis to high-affinity binding and catalysis. Chem Soc Rev. 44(2): 394–418, January 2015.
[49] Moghaddam, S., Yang, C., Rekharsky, M., Ko, Y. H., Kim, K., Inoue, Y., and Gilson, M. K.: New Ultrahigh Affinity Host-Guest Complexes of Cucurbit[7]uril with Bicyclo[2.2.2]octane and Adamantane Guests: Thermodynamic Analysis and Evaluation of M2 Affinity Calculations. Journal of the American Chemical Society. 133(10): 3570–3581, March 2011.
[50] Shetty, D., Khedkar, J. K., Park, K. M., and Kim, K.: Can we beat the biotin–avidin pair?: cucurbit[7]uril-based ultrahigh affinity host–guest complexes and their applications. Chem. Soc. Rev. 44(23): 8747–8761, 2015.
[51] Biedermann, F., Uzunova, V. D., Scherman, O. A., Nau, W. M., and De Simone, A.: Release of High-Energy Water as an Essential Driving Force for the High-Affinity Binding of Cucurbit[n]urils. J. Am. Chem. Soc. 134(37): 15318–15323, September 2012.
[52] Isaacs, L.: Stimuli Responsive Systems Constructed Using Cucurbit[n]uril-Type Molecular Containers. Acc. Chem. Res. 47(7): 2052–2062, July 2014.
[53] Vinciguerra, B., Zavalij, P. Y., and Isaacs, L.: Synthesis and Recognition Properties of Cucurbit[8]uril Derivatives. Org. Lett. 17(20): 5068–5071, October 2015.
[54] Lucas, D., Minami, T., Iannuzzi, G., Cao, L., Wittenberg, J. B., Anzenbacher, P., and Isaacs, L.: Templated Synthesis of Glycoluril Hexamer and Monofunctionalized Cucurbit[6]uril Derivatives. J. Am. Chem. Soc. 133(44): 17966–17976, November 2011.
[55] Ma, D., Zhang, B., Hoffmann, U., Sundrup, M. G., Eikermann, M., and Isaacs, L.: Acyclic Cucurbit[n]uril-Type Molecular Containers Bind Neuromuscular Blocking Agents In Vitro and Reverse Neuromuscular Block In Vivo. Angew. Chem. Int. Ed. 51(45): 11358–11362, November 2012.
[56] Ma, D., Hettiarachchi, G., Nguyen, D., Zhang, B., Wittenberg, J. B., Zavalij, P. Y., Briken, V., and Isaacs, L.: Acyclic cucurbit[n]uril molecular containers enhance the solubility and bioactivity of poorly soluble pharmaceuticals. Nat Chem. 4(6): 503–510, June 2012.
[57] Zhang, B. and Isaacs, L.: Acyclic Cucurbit[n]uril-type Molecular Containers: Influence of Aromatic Walls on their Function as Solubilizing Excipients for Insoluble Drugs. J. Med. Chem. 57(22): 9554–9563, November 2014.
[58] Gilberg, L., Zhang, B., Zavalij, P. Y., Sindelar, V., and Isaacs, L.: Acyclic cucurbit[n]uril-type molecular containers: Influence of glycoluril oligomer length on their function as solubilizing agents. Org. Biomol. Chem. 13(13): 4041–4050, 2015.
[59] Sigwalt, D., Moncelet, D., Falcinelli, S., Mandadapu, V., Zavalij, P. Y., Day, A., Briken, V., and Isaacs, L.: Acyclic Cucurbit[n]uril-Type Molecular Containers: Influence of Linker Length on Their Function as Solubilizing Agents. ChemMedChem. 11(9): 980–989, May 2016.
[60] Zhang, B., Zavalij, P. Y., and Isaacs, L.: Acyclic CB[n]-type molecular containers: Effect of solubilizing group on their function as solubilizing excipients. Org. Biomol. Chem. 12(15): 2413–2422, 2014.
[61] Ma, D., Zavalij, P. Y., and Isaacs, L.: Acyclic Cucurbit[n]uril Congeners Are High Affinity Hosts. J. Org. Chem. 75(14): 4786–4795, July 2010.
[62] She, N., Moncelet, D., Gilberg, L., Lu, X., Sindelar, V., Briken, V., and Isaacs, L.: Glycoluril-Derived Molecular Clips are Potent and Selective Receptors for Cationic Dyes in Water. Chem. Eur. J. pp n/a–n/a, August 2016.
[63] Ko, Y. H., Kim, E., Hwang, I., and Kim, K.: Supramolecular assemblies built with host-stabilized charge-transfer interactions. Chem. Commun. (13): 1305–1315, 2007.
[64] Barrow, S. J., Kasera, S., Rowland, M. J., del Barrio, J., and Scherman, O. A.: Cucurbituril-Based Molecular Recognition. Chem. Rev. 115(22): 12320–12406, November 2015.
[65] Urbach, A. R. and Ramalingam, V.: Molecular Recognition of Amino Acids, Peptides, and Proteins by Cucurbit[n]uril Receptors. Isr. J. Chem. 51(5-6): 664–678, May 2011.
[66] Connors, K. A.: Binding Constants. New York, NY, John Wiley & Sons, 1987.
[67] Masson, E., Ling, X., Joseph, R., Kyeremeh-Mensah, L., and Lu, X.: Cucurbituril chemistry: A tale of supramolecular success. RSC Adv. 2(4): 1213–1247, 2012.
[68] Márquez, C., Hudgins, R. R., and Nau, W. M.: Mechanism of Host-Guest Complexation by Cucurbituril. J. Am. Chem. Soc. 126(18): 5806–5816, May 2004.
[69] Biedermann, F., Nau, W. M., and Schneider, H.-J.: The Hydrophobic Effect Revisited—Studies with

Supramolecular Complexes Imply High-Energy Water as a Noncovalent Driving Force. Angew. Chem. Int. Ed. 53(42): 11158–11171, October 2014.
[70] 'il Saleh, N., Koner, A., and Nau, W.: Activation and Stabilization of Drugs by Supramolecular pKa Shifts: Drug-Delivery Applications Tailored for Cucurbiturils. Angewandte Chemie. 120(29): 5478–5481, July 2008.
[71] Nau, W. M., Florea, M., and Assaf, K. I.: Deep Inside Cucurbiturils: Physical Properties and Volumes of their Inner Cavity Determine the Hydrophobic Driving Force for Host–Guest Complexation. Isr. J. Chem. 51(5-6): 559–577, May 2011.
[72] Ghosh, I. and Nau, W. M.: The strategic use of supramolecular pKa shifts to enhance the bioavailability of drugs. Advanced Drug Delivery Reviews. 64(9): 764–783, June 2012.
[73] Gibb, C. L. D. and Gibb, B. C.: Binding of cyclic carboxylates to octa-acid deep-cavity cavitand. J Comput Aided Mol Des. 28(4): 319–325, November 2013.
[74] Sullivan, M. R., Sokkalingam, P., Nguyen, T., Donahue, J. P., and Gibb, B. C.: Binding of carboxylate and trimethylammonium salts to octa-acid and TEMOA deep-cavity cavitands. J Comput Aided Mol Des. pp 1–8, July 2016.
[75] Carnegie, R. S., Gibb, C. L. D., and Gibb, B. C.: Anion Complexation and The Hofmeister Effect. Angew. Chem. 126(43): 11682–11684, October 2014.